Guides to the Heart of the Spliceosome

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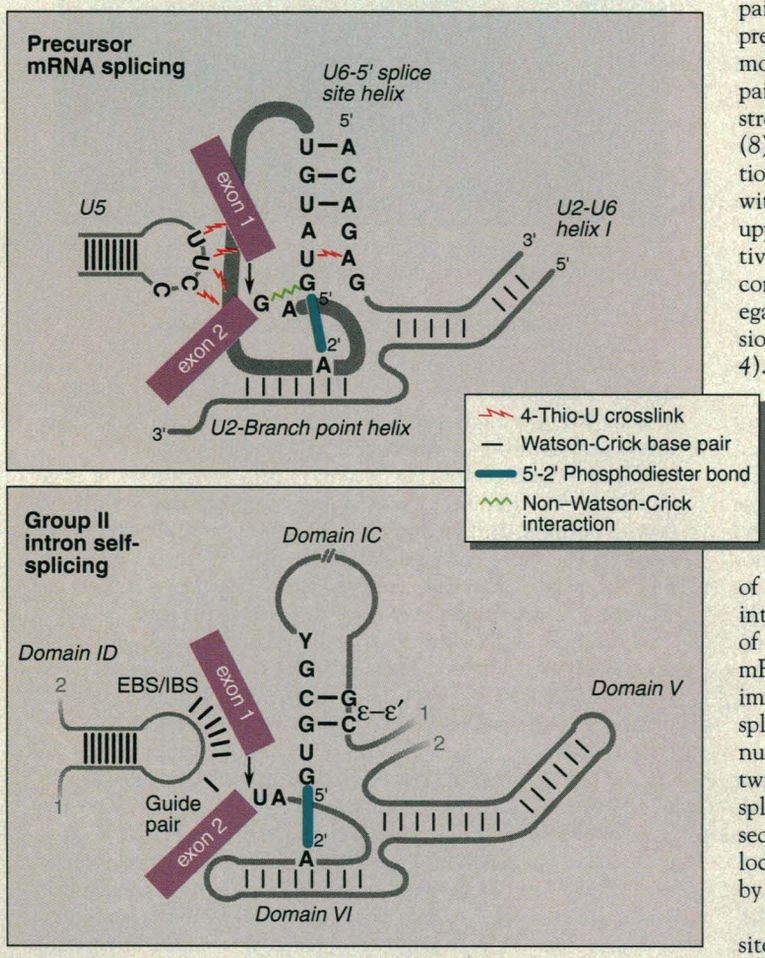
The Nobel Prize was awarded this year for the ground-breaking discovery in 1977 of intervening sequences (introns) that interrupt the coding regions (exons) of nuclear genes. It is therefore fitting that 1993 should mark major advances in elucidating the process by which introns are excised from newly synthesized RNA and the exons spliced to form mature messenger RNA (mRNA). Since 1977, five small nuclear RNAs (snRNAs) and an elaborate array of proteins have been identified as essential components of the spliceosome, the macromolecular complex responsible for precursor mRNA (pre-mRNA) splicing. Because splicing proceeds via a two-step mechanism analogous to the RNA-mediated autoexcision of another type of intron, the group II class, precursor mRNA splicing has long been suspected to be an RNA-catalyzed process (*1*). For both types of intron, the reaction pathway is initiated by the 2' hydroxyl group of an adenosine (located near the 3' end of the intron) attacking the 5' intron-exon splice junction, followed by joining of the two exons and release of the intron as a lariat structure. Despite significant progress over the past decade in elucidating RNA-RNA interactions critical for splicing (*2*), the precise mechanism by which the reactive groups are delivered to the catalytic center has remained elusive. In a remarkable convergence of genetics and biochemistry, three articles published in this issue of *Science* (*3*–*5*) provide compelling evidence that the splice sites are aligned during the reactions through a collaboration between U5 and U6 snRNAs.

An appealing model was proposed last year for juxtaposition of the 5' and 3' splice sites through simultaneous base-pairing of U1 snRNA with intron sequences and U5 snRNA with exon sequences to form a structure analogous to the Holliday genetic recombination intermediate (*6*). This structure seemed unlikely to be the substrate for catalysis, however, because the position of U1 pairing does not play a decisive role in specifying the site of nucleophilic attack. In contrast, U6 has emerged repeatedly as a likely component of the catalytic core of the spliceosome, beginning with its extraordinary conservation between yeast and mammals and culminating in the astonishing discovery that, at least in vitro, this snRNA can participate covalently in both steps of splicing (*7*). Recent crosslinking data placing U6 in proximity to the 5' junction at the time of the first of two transesterification reactions during splicing (*8*) prompted Lesser and Guthrie (*3*) and Kandels-Lewis and Séraphin (*4*) to test whether U6 might replace U1 in base-pairing with the intron in preparation for catalysis. Of two models previously proposed for pairing to sequences just downstream from the 5' splice site (*8*), the one involving evolutionarily invariant residues within U6 snRNA (see figure, upper part) had the most intuitive appeal and turns out to be correct, on the basis of an elegant series of genetic suppression experiments in yeast (*3*, *4*). Consistent with the idea that U6 aligns the 5' splice site for catalysis, compensatory base changes that improve Watson-Crick complementarity between the snRNA and the intron enhance the fidelity of the cleavage reaction. This interaction, the first example of direct scrutiny of the pre-mRNA by U6, underscores the importance of precision in the splicing pathway: The same nucleotides are recognized twice, first by U1 early in spliceosome assembly and subsequently by U6 to specify the location of nucleophilic attack by the branch point adenosine.

The idea that the 5' splice site is delivered to the catalytic center of the spliceosome by pairing to U6 snRNA is not only compatible with earlier crosslinking results (*8*), but is buttressed by the new data of Sontheimer and Steitz (*5*), which demonstrate contact between nucleotides in U6 and the intron that would be brought into proximity by the Watson-Crick pairing (see figure, upper part). The similarity in the location of this interaction relative to the 5' cleavage site, together with the persistence of the contact through the second step of splicing, suggests a functional analogy of the U6–5' splice site helix interaction to the ε-ε′ pairing in group II introns (compare the upper and lower parts of the figure). Notably, the apparent timing of U6 binding to the 5' splice site changes depending on the crosslinking agent employed (*5*, *8*). A logical interpretation of



Mechanism of intron excision and exon ligation during precursor messenger RNA and group II splicing. (Upper) Model for the catalytic core of the spliceosome poised for the second of the two transesterification reactions of splicing. Intron sequences, shaded line; 5' splice site sequence, *Saccharomyces cerevisiae* consensus; site of nucleophilic attack on the 3' splice site, arrow. **(Lower)** Model for the catalytic core of a group II self-splicing intron poised for the second transesterification reaction. The sequences shown are the consensus nucleotides for subgroup IIA from (*17*). Only a small portion of the group II structure is illustrated; the connectivity is indicated by numbers. IBS, intron binding site.

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this result, that the snRNA's mode of interaction with the substrate changes prior to the second transesterification reaction, is consistent with data on the stereochemical course of splicing by Moore and Sharp, which suggest that the spliceosome shifts between two active sites for the two steps of the reaction (*9*).

Because cleavage at the 5' splice site severs the covalent continuity of the splicing substrate, there must be some mechanism to allow the liberated 5' exon to be retained within the spliceosome so that its 3' hydroxyl group can serve as the nucleophile in the second step. U5 snRNA is an excellent candidate for this task, since genetic suppression experiments in yeast show that U5 can base-pair to nucleotides just upstream of the 5' splice site (*10*). To critically evaluate this model, it is essential to know the timing of this interaction relative to the transesterification reactions. This information is now supplied by the cleverly designed crosslinking experiments of Sontheimer and Steitz (*5*), which demonstrate that U5 snRNA makes contact with the nucleotide just upstream from the 5' splice site prior to the first cleavage. Even more telling, this interaction persists through both steps of splicing, clearly implicating U5 as the long-sought agent responsible for anchoring the free 5' exon. These data reinforce the idea that the U5 loop sequence is the spliceosomal counterpart of the exon binding site (EBS) in group II introns, which performs the analogous task (see figure).

The next problem faced by the spliceosome is how to orient the 3' splice site for the second phosphoester transfer. In group II introns, several interactions contribute to positioning the 3' junction for cleavage, including the "guide" pairing between a nucleotide just downstream from the 5' exon binding site and the first nucleotide of the 3' exon (*11*) (see figure, lower part). Genetic suppression experiments in yeast had suggested that a parallel interaction occurs during pre-mRNA splicing, because pairing of the sequence following the 5' exon binding site in the U5 loop to the 3' exon promotes the second step (*10*). The validity of this analogy is underscored by the crosslinking data of Sontheimer and Steitz (*5*), which show that the snRNA makes contact with the first nucleotide of the 3' exon only after the first step of splicing (see figure, upper part). Because introns interrupt codons for a variety of amino acids within eukaryotic pre-mRNAs, U5 must often carry out its role in aligning the two exons via noncanonical interactions, perhaps stabilized by proteins, whereas group II introns catalyze an intramolecular reaction and thus need only recognize the boundary sequences of the RNA in which they reside.

A second clue to how the 3' splice site is aligned for cleavage during pre-mRNA splicing emerged last year with the unexpected discovery that noncanonical pairing between the 5' and 3' bases of the intron is required for the second transesterification reaction (*12*). If this interaction occurred simultaneously with the newly defined Watson-Crick pairing between U6 and the 5' junction, it would bring an invariant nucleotide of the snRNA into proximity with the 3' splice site (figure, upper part). The partial suppression of splicing defects in 3' junction mutants upon mutating this residue of U6 implies that these nucleotides interact at least indirectly (*3*). The proximity of the U6 nucleotides that participate in the first and second steps of splicing suggests that, although different chemical groups may mediate catalysis of the two transesterification reactions, the active sites are likely to overlap substantially.

The new crosslinking and genetic suppression data presented in this issue of *Science* supply pieces of the spliceosome puzzle that were missing from a compelling model for the catalytic core previously proposed by Madhani and Guthrie (*13*). Through an elaborate series of genetic suppression experiments, they demonstrated that a helix strikingly similar to domain V of group II self-splicing introns, which is critical for catalysis (*14*), is formed through pairing of invariant residues in U2 and U6 snRNAs. This structure is flanked on one side by the U2 nucleotides that pair to the site of branch formation and on the other by the segment of U6 now implicated in splice site selection (see figure, upper part). Thus, the newly defined interactions extend the earlier model to explain, first, how the 5' junction might be delivered to the branch point for the first transesterification reaction and, second, how critical nucleotides in U6 could be juxtaposed with the 3' splice site during the second step. A comparable model, consistent with all currently available data, can be drawn to juxtapose the reacting nucleotides in group II self-splicing introns in preparation for the second step of splicing (see figure, lower part).

Do the proposed structural and functional analogies between group II and pre-mRNA splicing reflect descent from a common ancestor or, as has recently been suggested (*2*), are they the products of "chemical determinism," a common solution by unrelated RNAs to the problem of how to position sugar hydroxyls to carry out nucleophilic attack? Although this question can never be answered definitively, reliable three-dimensional structural models for both group II introns and the spliceosome should be illuminating. Even in the absence of this information, opportunities to evaluate the significance of the similarities noted to date should present themselves over the next few years. Particularly revealing will be the identification of binding sites for the metal ions that are likely to perform the actual work of catalysis in both reactions (*15*). Even now, the parallels extend well beyond apparently identical chemical mechanisms to include the use of remarkably similar helical domains to deliver the branch point nucleotide for nucleophilic attack at the 5' splice site, the positioning of exon sequences during both steps of the reaction by contiguous nucleotides within a hairpin loop, and recognition of sequences in corresponding locations on both sides of the cleavage sites through RNA-RNA interactions. Considering the amazing and ever increasing structural and mechanistic repertoire of RNA molecules (*16*), it seems improbable that two contemporary survivors of the "RNA world" could have independently arrived at such a similar assemblage of catalytic strategies. In fact, given that one reaction is carried out by multiple snRNAs acting in conjunction with several dozen polypeptides, while the other is catalyzed by cis-acting RNA sequences in the absence of protein, our ability to discern any resemblance at all suggests that we have, indeed, glimpsed the heart of the spliceosome.

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